

 

WHAT IS CLAIMED IS:

1. A method for identifying one or more nucleotides present at a polymorphic site on one or more alleles comprising the steps of:
 - a) obtaining an upper strand of target nucleic acids from the one or more alleles and a lower strand of target nucleic acids from the one or more alleles, wherein each strand comprises a polymorphic site;
 - b) hybridizing an upper strand primer that is complementary to the upper strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the upper strand of target nucleic acids so as to obtain one or more unpaired nucleotide bases to be identified on the upper strand, and hybridizing a lower strand primer that is complementary to the lower strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the lower strand so as to obtain one or more unpaired nucleotide bases to be identified on the lower strand;
 - c) exposing the hybridized upper and lower strand primers to a polymerization agent in a mixture comprising one or more nucleotides so that one or more primer extension products are formed if the one or more nucleotides in the mixture is complementary to the polymorphic site on the upper strand or lower strand of target nucleic acids; and
 - d) separating any one or more primer extension products from unextended primers so as to identify the polymorphic site on the one or more alleles.
2. A method according to claim 1, wherein the mixture comprises one or more chain terminating nucleotides.
3. A method according to claim 2, wherein the one or more chain terminating nucleotides with the same detectable characteristic.
4. A method according to claim 2, wherein the one or more chain terminating nucleotides are selected from the group consisting of dideoxynucleotides and acyclonucleotides.
5. A method according to claim 1, wherein the mixture comprises at least four different chain terminating nucleotides.

6. A method according to claim 5, wherein the mixture comprises at least four different chain terminating nucleotides, at least two different chain terminating nucleotides have the same detectable characteristic.
7. A method according to claim 6, wherein at least two different terminating nucleotides are distinguished from each other by a detectable characteristic selected from the group consisting of inherent mass, electric charge, electron spin, mass tag, radioactive isotope, dye, bioluminescent molecule, chemiluminescent molecule, nucleic acid molecule, hapten molecule, protein molecule, light scattering/phase shifting molecule, or fluorescence molecule.
8. A method according to claim 1, wherein the upper and lower strand primers each contain a unique tag at the 5' end of each primer.
9. A method according to claim 8, wherein each unique tag comprises a sequence that is capable of hybridizing with a complementary sequence at known positions on a solid support.
10. A method according to claim 9, wherein each unique tag comprises from about 8 to about 40 nucleotides.
11. A method according to claim 9, wherein the solid support is selected from the group consisting of silica gel, silicon, glass, polystyrene, nylon, polypropylene, nitrocellulose or CPG.
12. A method according to claim 8, wherein each unique tag comprises a sequence that is capable of hybridizing with a complementary sequence at known positions on an array.
13. A method according to claim 1, wherein the upper and lower strand of target nucleic acids are genomic or mitochondrial DNA.

DRAFT
PCT
US
33
33
33
33
33
33
33
33

14. A method according to claim 1, wherein the polymorphic site is selected from the group consisting of a single nucleotide polymorphism, an insertion, a deletion, a re-arrangement, or a repetitive sequence.

15. A method according to claim 1, wherein the method is performed in solution phase in one or more single wells.

16. A method for identifying one or more nucleotides present at a polymorphic site on one or more alleles comprising the steps of:

a) obtaining an upper strand of target nucleic acids from the one or more alleles and a lower strand of target nucleic acids from the one or more alleles; wherein each strand comprises the polymorphic site;

b) hybridizing an upper strand primer that is complementary to the upper strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the upper strand of target nucleic acids so as to obtain one or more unpaired nucleotide bases to be identified on the upper strand, and hybridizing a lower strand primer that is complementary to the lower strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the lower strand so as to obtain one or more unpaired nucleotide bases to be identified on the lower strand; the upper and lower strand primers each have a unique tag at the 5' end capable of binding to known positions on a solid support;

c) exposing the hybridized upper and lower strand primers to a polymerization agent in a mixture comprising one or more nucleotides so that one or more primer extension products are formed if the one or more nucleotides in the mixture is complementary to the polymorphic site on the upper strand or lower strand of target nucleic acids;

d) contacting the solid support with the mixture so as to cause each unique sequence tag to bind to known positions on the solid support; and

e) detecting each bound primer, wherein the positions of the primers on the solid support in conjunction with any one or more primer extension products allows identification of the polymorphic site on the one or more alleles.

17. A method according to claim 16, wherein the mixture comprises one or more chain terminating nucleotides.

18. A method according to claim 17, wherein the one or more chain terminating nucleotides have the same detectable characteristic.
19. A method according to claim 17, wherein the one or more chain terminating nucleotides are selected from the group consisting of dideoxynucleotides or acyclonucleotides.
20. A method according to claim 16, wherein the mixture comprises at least four different chain terminating nucleotides.
21. A method according to claim 20, wherein the mixture comprises at least four different chain terminating nucleotides, at least two different chain terminating nucleotides have the same detectable characteristic.
22. A method according to claim 21, wherein at least two different terminating nucleotides are distinguishable from each other by a detectable characteristic selected from the group consisting of inherent mass, electric charge, electron spin, mass tag, radioactive isotope, dye, bioluminescent molecule, chemiluminescent molecule, nucleic acid molecule, hapten molecule, protein molecule, light scattering/phase shifting molecule, or fluorescence molecule.
23. A method according to claim 16, wherein each unique tag comprises a nucleic acid sequence that is capable of hybridizing with a complementary sequence at known positions on a solid support.
24. A method according to claim 23, wherein each unique tag comprises from about 8 to about 40 nucleotides.
25. A method according to claim 23, wherein the solid support is selected from the group consisting of silica gel, silicon, glass, polystyrene, nylon, polypropylene, nitrocellulose or CPG.

26. A method according to claim 16, wherein each unique tag comprises a nucleic acid sequence that is capable of hybridizing with a complementary sequence at known positions on an array.
27. A method according to claim 16, wherein the upper and lower strand of target nucleic acids are genomic or mitochondrial DNA.
28. A method according to claim 16, wherein the polymorphic site is a single nucleotide polymorphism, an insertion, a deletion, a re-arrangement, or repetitive sequence.
29. A method according to claim 16, wherein step (c) is performed in solution phase in one or more single wells.
30. A method for identifying one or more nucleotides present at a polymorphic site on the one or more alleles comprising the steps of:
- a) obtaining an upper strand of target nucleic acids from the one or more alleles and a lower strand of target nucleic acids from the one or more alleles, wherein each strand comprises the polymorphic site;
 - b) hybridizing an upper strand primer that is complementary to the upper strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the upper strand of target nucleic acids so as to obtain an unpaired nucleotide base to be identified at the polymorphic site on the upper strand, and hybridizing a lower strand primer that is complementary to the lower strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the lower strand so as to obtain an unpaired nucleotide base to be identified at the polymorphic site on the lower strand; wherein the upper and lower primers each have a unique tag at the 5' end capable of binding to known positions on a solid support; and
 - c) exposing the hybridized upper and lower strand primers to a polymerization agent in a mixture comprising at least four different terminating nucleotides so as to form primer extension products wherein the primers are extended bidirectionally when the terminating nucleotide in the mixture is complementary to the polymorphic site on the upper strand or lower strand of target nucleic acids; wherein at least two different terminating nucleotides have the same detectable characteristic;

- d) contacting the solid support with the mixture so as to cause each unique sequence tag to bind to known positions on the solid support; and
- e) detecting each bound primer, wherein the positions of the primers on the solid support in conjunction with any detectable characteristic allows identification of the polymorphic site on the one or more alleles.
31. A method according to claim 30, wherein the four different terminating nucleotides are selected from the group consisting of dideoxynucleotides or acyclonucleotides.
32. A method according to claim 30, wherein the detectable characteristic is selected from the group consisting of inherent mass, electric charge, electron spin, mass tag, radioactive isotope, dye, bioluminescent molecule, chemiluminescent molecule, nucleic acid molecule, hapten molecule, protein molecule, light scattering/phase shifting molecule or fluorescence molecule.
33. A method according to claim 30, wherein each unique tag comprises a nucleic acid sequence that is capable of hybridizing with a complementary sequence at known positions on a solid support.
34. A method according to claim 30, wherein each unique tag comprises from about 8 to about 40 nucleotides.
35. A method according to claim 30, wherein the solid support is selected from the group consisting of silica gel, silicon, glass, polystyrene, nylon, polypropylene, nitrocellulose or CPG.
36. A method according to claim 30, wherein each unique tag comprises a nucleic acid sequence that is capable of hybridizing with a complementary sequence at known positions on an array.
37. A method according to claim 30, wherein the upper and lower target nucleic acids are genomic or mitochondrial DNA.

SEARCHED
INDEXED
SERIALIZED
FILED

38. A method according to claim 30, wherein step (c) is performed in solution phase on one or more single wells.
39. A method for identifying one or more nucleotides present at a polymorphic site on one or more alleles comprising the steps of:
- obtaining an upper strand of target nucleic acids from the one or more alleles and a lower strand of target nucleic acids from the one or more alleles, wherein each strand comprises the polymorphic site;
 - hybridizing an upper strand primer that is complementary to the upper strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the upper strand of target nucleic acids so as to obtain one or more unpaired nucleotide bases to be identified on the upper strand, and hybridizing a lower strand primer that is complementary to the lower strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the lower strand so as to obtain one or more unpaired nucleotide bases to be identified on the lower strand;
 - exposing the hybridized upper and lower strand primers to a polymerization agent in a mixture comprising one or more nucleotides so that one or more primer extension products are formed if the one or more nucleotides in the mixture are complementary to the polymorphic site on the upper strand or lower strand of target nucleic acids wherein the primers are extended bidirectionally; and
 - separating any one or more primer extension products from unextended primers so as to identify the polymorphic site on the one or more alleles.
40. A method according to claim 5, wherein the mixture comprises at least four different chain terminating nucleotides, at least one chain terminating nucleotide has a label.
41. A method according to claim 20, wherein the mixture comprises at least four different chain terminating nucleotides, at least one chain terminating nucleotide has a label.
42. A method according to claim 1, wherein the upper and lower strand primers are immobilized to a solid support.

43. A method according to claim 1, wherein the upper strand target and lower strand target nucleic acids are amplified prior to hybridization.
44. A method according to claim 1, wherein the method is performed on multiple upper and lower strand targets nucleic acids simultaneously.
45. A method according to claim 1, wherein the method is performed on two alleles.
46. A method according to claim 1, wherein step (e) is performed by single channel detection.
47. A method for identifying one or more nucleotides present at a polymorphic site on one or more alleles comprising the steps of:
- a) obtaining an upper strand of target nucleic acids from the one or more alleles and a lower strand of target nucleic acids from the one or more alleles, wherein each strand comprises a polymorphic site;
 - b) hybridizing an upper strand primer that is complementary to the upper strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the upper strand of target nucleic acids so as to obtain one or more unpaired nucleotide bases to be identified on the upper strand, and hybridizing a lower strand primer that is complementary to the lower strand of target nucleic acids at a region immediately adjacent to the polymorphic site on the lower strand so as to obtain one or more unpaired nucleotide bases to be identified on the lower strand;
 - c) exposing the hybridized upper and lower strand primers to a polymerization agent in an extension mixture comprising one or more nucleotides so that one or more primer extension products are formed if the one or more nucleotides in the extension mixture is complementary to the polymorphic site on the upper strand or lower strand of target nucleic acids; and
 - d) detecting in the extension mixture one or more nucleotides not incorporated into the one or more primer extension products so as to identify the one or more nucleotides present at the polymorphic site.